PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 37/64, 31/56

A1

(11) International Publication Number: WO 92/06706

(43) International Publication Date: 30 April 1992 (30.04.92)

(21) International Application Number: PCT/US91/06847

(22) International Filing Date: 26 September 1991 (26.09.91)

(30) Priority data:

598,241	16 October 1990 (16.10.90)	US
643,727	18 January 1991 (18.01.91)	US
683,620	11 April 1991 (11.04.91)	US

(71)(72) Applicants and Inventors: LEZDEY, John [US/US]; 976 Kingston Drive, Cherry Hill, NJ 08034 (US). WACHTER, Allan [US/US]; 9822 South Grandview, Tempe, AZ 85284 (US).

(74) Common Representative: LEZDEY, John; Suite 400, 400 Market Street, Philadelphia, PA 19106 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), BF (OAPI patent), BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), ML (OAPI patent), MR (OAPI patent), NL (European patent), NO, PL, SE (European patent), SN (OAPI patent), SU+,TD (OAPI patent), TG (OAPI patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TREATMENT OF INFLAMMATION

(57) Abstract

A method for the prophylaxis or direct treatment of mast cell implicated diseases or injuries in a patient which comprises administering to the site of the disease or injury an effective amount of at least one serine protease inhibitor, its salts, derivatives or analogs which bind with the mediators of mast cells or T-cells.

+ See back of page

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU+**	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

TREATMENT OF INFLAMMATION

Field of the Invention

The present invention relates to a method and composition for treating mammals afflicted with mast cell implicated disease. More particularly, the present invention relates to the direct or prophylaxis treatment of certain mast cell implicated diseases, particularly inflammatory conditions in patients, by administering serine protease inhibitors, their analogs, salts or derivatives. There is particularly provided topical compositions for treating the symptoms of inflammatory skin conditions, compositions for treating pulmonary inflammation by inhalation therapy and compositions for treating allergic rhinitis.

Background of the Invention

Prior to the present invention it was generally believed that serine protease inhibitors could be used only to supplement a deficiency occurring as a result of a genetic defect or a chemically produced deficiency resulting from an event such as smoking. Moreover, no consideration was previously given for directly controlling diseases in which mast cells are implicated by administering serine protease inhibitors when serum levels of proteases or protease inhibitors are normal. Mast cells have been found to be implicated in diseases and events such as allergic and non-allergic rhinitis, nasal polyposis, atopic dermatitis, including psoriasis, contact dermatitis, pancreatitis, emphysema, asthma, colitis, Crohn's Disease, wound healing, cluster headaches, coronary artery spasm, rheumatoid arthritis etc.

Inflammation is a non-specific response of tissues to diverse stimuli or insults and results in release of a variety of materials at the site of inflammation that induce pain. It is now recognized that mast cells are implicated in the pathophysiology

of inflammatory skin conditions as well as in other physiological disorders. Mast cells provide the greatest source of histamines in acute inflammation. Mast cells have also been noted in hypertropic scars.

It is now recognized that in a certain injury or a disease neutrophils, mast cells, T-cells and their mediators induce an inflammatory state resulting in a localized imbalance of elevated serine proteases with a concominant deficiency of their naturally occurring inhibitors despite normal serine protease inhibitor serum levels. Mast cells are critical in recruiting the cells (eosinophils, basophils and neutrophils) involved in the late phase reaction (LPR). Mast cell and neutrophile mediators appear to have a central role in the LPR. Monocytes through the release of cytokines, interleukin -1,6 and tumor necrosis factor further amplify the LPR. Platelet activating factor, a mediator from mast cells, neutrophils and platelets is a potent bronchoconstrictor. Histamines are also released by the degranulation of mast cells as well as leukotriene T4 (LTB4) which play an important role in asthma. IgE upon activation by an antagonist causes degranulation of mast cells. Alpha 1-antitrypsin, as well as alpha 1-antichymotrypsin inhibits the mediators of mast cells and neutrophils, and also regulates IgE biosynthesis. The T-cell lymphokine glycosylation enhancing factor (GEF) is a serine protease that has been shown to enhance IgE response. The serine protease inhibitors decrease mast cell mediator release by inhibiting local IgE biosynthesis and T-cell lymphokine production. Serine proteases not only activate kinins and complements but also mediate tissue necrosis. The serine proteases, elastase and cathepsin G, have been shown to stimulate the production of platelet activating factor and LTB4.

Eosinophils and neutrophils are prominent in inflammatory lesions due to the potent

chemoattractants released by mast cells.

Neutrophils are a main source of serine elastase and cathepsin G which are important in the tissue damage of inflammation, especially in rheumatoid arthritis.

The most direct approach to therapy of inflammatory skin conditions appears to be a direct attack at the site of inflammation of the mediators of inflammation and pain and the reduction of those neutrophilic derivatives which can cause damage to the growth of new tissue during the healing process.

Alpha 1-antichymotrypsin is a plasma protease inhibitor synthesized in the liver. It is a single glycopeptide chain of approximately 68,000 daltons and belongs to a class of serine protease inhibitors with an apparent affinity toward chymotrypsin-like enzymes.

Alpha 2-macroglobulin is a glycoprotein containing 8-11% carbohydrate which can be isolated from plasma by gel filtration chromatography.

Alpha 1-proteinase inhibitor (alpha 1-antitrypsin) is a glycoprotein having a molecular weight of 53,000 determined by sedimentation equilibrium centrifugation. The glycoprotein consists of a single polypeptide chain to which several oligosaccharide units are covalently bonded. Human alpha 1-proteinase inhibitor has a role in controlling tissue destruction by endogenous serine proteinases. A genetic deficiency of alpha-1-proteinase inhibitor, which accounts for 90% of the trypsin inhibitory capacity in blood plasma, has been shown to be associated with the premature development of pulmonary emphysema. The degradation of elastin associated with emphysema probably results from a local imbalance of elastolytic enzymes and the naturally occurring tissue and plasma proteinase inhibitors. Alpha-1-proteinase inhibitor inhibits human pancreatic and leukocyte elastases. See Pannell et al, Biochemistry. 13, 5339

(1974); Johnson et al, Biochem. Biophys. Res. Commun., 72 33 (1976); Del Mar et al, Biochem. Biophys. Res. Commun., 88, 346 (1979); and Heimburger et al, Proc. Int. Res. Conf. Proteinase Inhibitors. 1st, 1-21 (1970).

The article of Groutas entitled "Inhibitors of Leukocyte Elastase and Leukocyte Cathepsin G Agents for the Treatment of Emphysema and Related Ailments" medical Research Reviews, Vol. 7, No. 7, 227-241 (1987), discloses the role of eglin, elastinal 1 and elasnin in emphysema.

U.S. Pat. No. 4,732,973 to Barr et al discloses typical analogs of serine protease inhibitors which may be used in the present invention.

U.S. Patent No. 4,916,117 to Lezdey et al discloses the treatment of pulmonary inflammation with microcrystalline alpha-1-antichymotrypsin.

It is understood that the term "serine protease inhibitors" as used herein refers to the inhibitors derived from a particular species and inhibits the proteases of the same species. However, human serine protease inhibitors may be used in veterinary products but not visa versa.

Summary of the Invention

The present invention relates to a method for treating inflammatory conditions in patients with mast cell implicated diseases by the administration of serine protease inhibitors, their analogs, salts or derivatives, alone or in combination with one or more other serine protease inhibitors which have a specific activity for mast cells or the proteases derived therefrom such as cathepsin-G, elastase, human mast cell chymase, kinins, and T-cell proteases or their precursors in a suitable pharmaceutical composition.

Serine protease inhibitors have been found to play a major role in the direct inactivation of the mediators of inflammation so that the normal wound

healing process can be accelerated without interference from the excess of materials released at the site of inflammation. The almost immediate disappearance of pain and itch indicates that there can be a control of the kinins as well. A cocktail of serine protease inhibitors would therefore be useful to deactivate those mediators of inflammation which may not yet be recognized but are found in association with a particular inflammatory disease.

It is now recognized that in certain injuries or diseases, neutrophils, mast cells, T-cells and their mediators induce an inflammatory state resulting in a localized imbalance of elevated serine protease with a concomitant deficiency of their naturally occurring inhibitors despite normal serine protease inhibitor serum levels. Mast cells are critical in recruiting the cells (eosinophils, basophils and neutrophils) involved in the late phase reaction (LPR). Mast cell and neutrophil mediators appear to have a central role in the LPR. Monocytes through the release of cytokines, interleukin -1,6 and tumor necroses factor further amplify the LPR. Platelet activating factor, a mediator from mast cells, neutrophils and platelets is a potent bronchoconstrictor. Histamines are also released by the degranulation of mast cells as well as leukotriene T4 (LTB4) which play an important role in asthma. IgE upon activation by an antagonist causes degranulation of mast cells. Alpha 1-antitrypsin, as well as alpha 1-antichymotrypsin inhibits the mediators of mast cells and neutrophils, and also regulates IgE biosynthesis. The T-cell lymphokine glycosylation enhancing factor (GEF) is a serine protease that has been shown to enhance IgE response. By also inhibiting GEF there is a two level inhibition in the inflammatory cycle. The serine protease inhibitors decrease mast cell mediator release by inhibiting local IgE biosynthesis and T-cell

lymphokine production. Serine proteases not only activate kinins and complements but also mediate tissue necrosis. The serine proteases, elastase and cathepsin G, have been shown to stimulate the production of platelet activating factor and LTB4.

Alpha 1-antichymotrypsin is important because it binds with basophils which have a high content of cathepsin G. By controlling the basophils there is also control of the histamine release factor.

As presently found, serine protease inhibitors are useful in the treatment of burn patients which not only experience pain and itch but have a problem in controlling the laydown of organized collagen because of elastase and cathepsin G; serine protease inhibitors particularly alpha 1-antitrypsin and alpha 1-antichymotrypsin, permit the rapid growth of normal skin without degranulation.

The administration of serine protease inhibitors appears to be a viable alternative to the administration of steroids to reduce inflammation and to treat inflammatory skin conditions not treatable with steroids or to reduce the steriod requirement. However, the combination with a cortiscosteroid has been found to provide a synergistic effect.

It has now been found that controlling the amount of the destructive enzymes at the site of inflammation can prevent proliferation of the disease, prevent associated tissue damage and promote healing. It has also been found that the administration of serine protease inhibitors which inactivate destructive proteases alone provide a major control of the symptoms of the disease or burns. However, since the cause of disease may be a result of more than one factors, the use of more than one protease inhibitor provides a better chance of success for early remission of the symptoms and for a prophylactic control of the symptoms associated with the disease.

Serine protease inhibitors, for example, alpha 2-macroglobulin, alpha 1-antichymotrypsin and C-reactive protein (CRP), when administered to the site of inflammation provides a reduction in swelling, pain and stiffness.

For chronic cases of dermatitis, a cocktail of serine protease inhibitors is preferably administered at the site of inflammation. The treatment can be followed with the addition of an appropriate steroid or antibiotic. There is a synergistic effect when the serine protease inhibitor is used in combination with a corticosteroid.

Among the corticosteroids which may be used in the present invention are triamcinolone acetonide, flurandrenolide, prednisone, amcinonide, dexamethasone, betamethasone valerate, halocinonide, clocortolone, hydrocortisone valerate, and the like.

Serine protease inhibitors have been found to play a major role in the direct inactivation of the mediators of inflammation so that the normal wound healing process can be accelerated without interference from the excess of materials released at the site of inflammation. The almost immediate disappearance of pain and itch indicates that there can be a control of the kinins as well. Serine protease inhibitors, their analogs, salts or derivatives, appears to provide the quickest healing of psoriatic lesions when used in combination with a corticosteroid.

As presently found, serine protease inhibitors are useful in the treatment of chronic psoriasis patients which not only experience pain and itch but have a problem in controlling the laydown of organized collagen because of elastase and cathepsin G; serine protease inhibitors permit healing and the growth of normal skin. The presence of the steroids enhance the healing and promote a more rapid skin growth which is

initiated by the serine protease inhibitors.

The serine protease inhibitors which are contemplated in the present invention are any of the inhibitors, their analogs, derivatives or salts of the human type which can inhibit mast cells or bind with any one or more of the protease derived from eosinophils, basophils and/or neutrophils such as elastase, cathepsin-G, tryptase, chymase, kinins, kallikrein, tumor necrosis factor, chymotrypsin, collagenase, inhibit IgE production and the like.

The serine protease inhibitors included in the present invention are human alpha 1-antichymotrypsin, alpha 1-antitrypsin, alpha 2-macroglobulin, eglin, elastinal 1, elasnin 3, eglin 2, C-reactive protein, beta 1-antigellagenase, serine amyloid A protein, alpha cysteine protease inhibitors, inter-alpha-trypsin inhibitor, secretory leucocyte protease inhibitor, bronchial mucous inhibitor, and C-1-inhibitor. The inhibitors of the invention may be natural or prepared by recombinant means. The recombinant may be glycosylated.

The use of alpha 1-antitrypsin and alpha 1-antichymotrypsin have been especially useful in the treatment of the various inflammatory skin conditions including those which are induced by autoimmune disease, virus and bacterial infections. The serine protease inhibitors have also been found to cause vasoconstriction, which in inflammation, decreases swelling and redness and to eliminate pain and itching. This feature is especially useful in burns and atopic dermatitis.

Alpha 1-antitrypsin has also been found especially useful in the treatment of bronchial and topical inflammatory conditions because of its association with elastase. However, it is preferably used in combination with alpha 1-antichymotrypsin which is not deactivated by oxidants.

The drugs of the invention may be derived from human blood or prepared by cloning, by conventional techniques utilizing an oligonucleotide probe or antibody probe, and the like. The recombinant gene product of the invention is especially useful since it is free of contaminating viruses when produced.

The analogs, salts and derivatives may be formed utilizing conventional techniques associated with other proteins without effecting the utility of the compound. There may be prepared the alkali metal salts, acid-addition salts, and esters similar to other proteins or peptides.

Some inflammation conditions are not immediately identifiable as to source and the factors which are involved to produce the different symptoms are not readily apparent. Therefore, it is desirable to administer in some case a combination or cocktail of serine protease inhibitors to provide a broad spectrum of drugs which can provide rapid relief of the different symptoms of inflammation. The most effective combination is alpha 1-antichymotrypsin and alpha 1-antitrypsin and/or alpha 2-macroglobulin. Preferably, the combination is administered in a ratio of 1:1:1: to 3:2:1: either in a single unit or in separate dosage form.

When topically applied, a serine protease inhibitor such as alpha 1-antitrypsin in suitable composition form is useful in the treatment of burns and inflammatory skin diseases such as psoriasis, eczema, acne, and the like. It has been demonstrated that treatment with alpha 1-antichymotrypsin together with α1-antitrypsin has reduced pain when applied to skin lesions.

The use of a non-aqueous lipid miscible carrier, for example, such as prepared with liposomes are particularly advantageous since they provided improved activity at the treatment sites.

The compositions of the invention are preferably administered to patients showing an increase in IgE through a patch or serum test. That is, the patient shows a positive allergic condition. These allergic patients having asthma respond quickly to therapy with alpha 1-antitrypsin when administered by inhalation form.

The present invention also provides a method for the prophylactic and direct treatment of patients suffering from allergic rhinitis and the symptoms thereof. In accordance with the invention, there is nasally administered to the patient an effective amount of a serine protease inhibitor, its analog, derivative or salt in a suitable pharmaceutically acceptable carrier. The serine protease inhibitors, analog, derivative or salt is one which is capable of binding with a protease in pollen, a protease derived from mast cells, neutrophils or T-cells or decreasing the degranulation of mast cells by inhibiting antagonists such as GEF.

Preferably, the serine protease inhibitor is administered in an aqueous solution comprising 0.1 to 4.5% by weight of the inhibitor. A greater amount can be used but is generally not required.

The serine protease inhibitor binds with a stimulator of IgE synthesis or an inhibitor of mast cell degranulation. These inhibitors further prevent protease from activating complement and kinins which cause the discomfiture associated with the disease.

The term "allergic rhinitis" is understood to include rhinitis medicamentosa, rhinitis sicca and atrophic rhinitis. Preferable are the serine protease inhibitors which have a specific inhibiting activity of mast cells and binding with the proteases derived therefrom such as cathepsin-G, elastase, human mast cell chymase, kinins, and the like. The inhibiting activity may be direct or indirect. It has now been

found that controlling the amount of mast cells and their mediators inherently controls the amount of the enzymes at the site of inflammation and prevents proliferation of the condition. Serine protease inhibitors or acute phase reactants, when administered to the site of inflammation provides a reduction in swelling of the sinuses.

In the treatment of burns, a 20% solution of a serine protease inhibitor such as α 1-antitrypsin, alone or in combination with other serine protease inhibitors, in sterile water or saline solution, may be sprayed on the patient or the burn area may be wrapped in wet bandages. A wound healing or skin growth factor may be included. The treatment provides immediate relief of pain. The patient may then be treated with the solution daily until the healing process is normal. Depending upon the severity of the burns, the patient may be further treated with other medications to prevent infection.

The treatment of rheumatoid arthristis can be performed by injection and/or by topical application such as utilizing an occlusive dressing and an aqueous composition of the drug.

The following examples further illustrate the practice of this invention, but are not intended to be limiting thereof. It will be appreciated that the selection of actual amounts of specific serine protease inhibitors to be administered to any individual patient (human or animal) will fall within the discretion of the attending physician and will be prescribed in a manner commensurate with the appropriate dosages will depend on the stage of the disease and like factors uniquely within the purview of the attending physician.

EXAMPLE I

A topical cream was prepared as follows:

A. The following mixture was prepared:

α_1 -antitrypsin	1.0 g
Olive oil	5.0 g
Cetanol	2.0 g
Stearic acid	5.0 g
Glycerin aliphatic acid ester	12.0 g
Tween 60	0.5 g

B. The following mixture was also prepared:

Propylene glycol	0.5 g
Methyl paraben	0.1 g
Propyl paraben	0.02 g
Purified water	to 100 g

in total

The mixture of parts A and B were blended together by conventional means to give a total of 100 g. of 100% by weight topical cream which could be utilized for treatment of acne, eczema, psoriasis, or other inflammatory dermatological conditions. If desired secretory leucocyte protease inhibitor and/or alpha 2-macroglobulin as well as a corticosteroid may be added in an amount of 1.0 g to part A.

EXAMPLE II

An olegenous anhyrous ointment was prepared with the following composition:

Composition	%
α_1 -antitrypsin	1.0
Soy phosphatide	4.0
Plastibase 50W	94.975
Butylated hydroxytoluene	0.025
	100.00

If desired, in lieu of alpha 1-antitrypsin as the active principal, there may utilized the combination of alpha 1-antichymotrypsin and alpha 1-antitrypsin. Other non-aqueous lipid miscible carriers may also be utilized. The composition may be used in combination

with a topical corticosteroid.

EXAMPLE III

1000 mg of PROLASTIN, a composition sold by Cutter Biological, Miles Inc., comprising about 70% α_1-antitrypsin and about 10-18% α_1-antichymotrypsin was dissolved in 50 ml of saline solution. A patient suffering from atopic dermatitis with swelling and open lesions of the hand was treated by immersing the hand in the solution. Pain disappeared within 6-10 minutes of treatment. Treatment was continued for 1 hour. The redness and swelling disappeared after 1 hour. Twenty four hours after the treatment the lesions were healing without treatment with any other drugs.

A similar composition was utilized as an otic wash for cats with ear infections followed by the administration of a steroid.

Example IV

A suitable cream for topical use was prepared by admixing 43 g of PROLASTIN from Cutter Biological Laboratories, with 6 ml of water and 1000 g of a balm available under the trademark AQUAPHOR, sold by Beiesdorf Inc., Norwalk CT. AQUAPHOR comprises a mixture of petrolatum, minerial oil, wax and wool wax alcohol.

The cream is useful for minor irritations and in the prophylaxis treatment of inflammatory skin conditions.

Example V

In the treatment of colitis a 20% solution with alpha 1-antitrypsin may be prepared and administered as an enema.

A similar result will be found with an secretory leucocyte protease inhibitor.

Example VI

1000 mg of PROLASTIN, a composition sold by Cutter Biological, Miles Inc., comprising about 70% α_1-antitrypsin and about 10-18% α_1-antichymotrypsin was dissolved in 50 ml of saline solution. A patient suffering from psoriasis with swelling and open lesions of the hand was treated by immersing the hand in the solution. The patient was previously treated only with steroids for 3 years without success. Pain disappeared within 6-10 minutes of treatment. Treatment was continued for 1 hour. After treatment wiht PROLASTIN, 0.1% mometasone furoate was applied. The treatment was continued with alternate day application of PROLASTIN and daily applications of mometasone furoate.

After three weeks all of the symptoms of psoriasis disappeared and 90% of the skin rash disappeared.

The same procedure is effective in treating the symptoms of psoriatic arthritis.

Example VII

A suitable cream for topical use was prepared by

admixing 43 g of PROLASTIN from Cutter Biological Laboratories, with 6 ml of water and 1000 g of a balm available under the Trademark AQUAPHOR, sold by Beiesdorf Inc., Norwalk CT. AQUAPHOR comprises a mixture of petrolatum, minerial oil, wax and, wool wax alcohol.

The cream is useful for the prophylaxis treatment of psoriasis.

Example VIII

Microcrystalline alpha-1-antitrypsin is suspended in oleic acid and added into a metering aerosol cannister together with trichloromonofluoromethane and dichlorodifluoromethane so that the unit has a molecular proportion of alpha-1-antitrypsin to the propellant between 3:1 and 3:2. The unit delivers a quantity of drug equivalent to 42 mcg. The composition can be used in the treatment of asthma.

Example IX

Microcrystalline alpha-1-antitrypsin and alpha-1-antitrypsin is suspended in oleic acid and added into a metering aerosol cannister together with trichloromonofluoramethane and dichlorodifluoromethane so that the unit has a molecular proportion of drug to the propellant between 3:1 and 3:2.

Example X

A composition for use in treating allergic rhinitis was prepared from the following ingredients.

Ingredient	% wt

α_1-antitrypsin	0.1
10% saline solution	99.8
antioxidant	0.1

Example XI

A 10 ml solution which is effective for use as a nasal spray or nose drops was prepared with the following ingredient:

Ingredient	% wt
α_1-antitrypsin	2.5 mg
α_1-antichymotrypsin	2.5 mg
sorbitol solution	571.0 mg
vitamin E	2.0 mg
purified water	q.s.

Example XII

A 0.15% by weight solutio of PROLASTIN, a composition sold by Cutter Biological, Miles Inc., comprising about 70% α_1-antitrypsin and about 10-18% α_1-antichymotrypsin with a 10% saline solution. The solution prepared could be packaged for use as a nasal spray or as nose drops.

Example XIII

A pilot study was performed which consisted of a non-blinded trial using α_1-PI at a concentration of 20mg/ml in an aqueous solution in an alternate day schedule in conjunction with a 1% cream of α_1-PI (Stage I) and a 5% cream of α_1-PI for maintenance therapy (Stage II). Prior to enrollment in this trial all 6 patients failed to respond to high potency topical steroids. Safety was gauged by careful clinical monitoring of subjective complaints, objective findings of erythema, edema and serial measurements of blood chemistries and complete blood counts. Wound healing was documented by serial photography. Written informed consent was obtained from each patient.

All six patients showed significant clinical improvement within 6 to 21 days of initiation of alternate-day therapy. α_1-PI stopped pain, pruritis and promoted tissue healing without scarring in all six patients. No adverse side effects of therapy were documented by clinical history, physical exam or by blood studies after 120 days of therapy. The results

is seen in Table 1.

Pt. #	Age /sex	Clinical Manifestions	Duration of Illness/ Previous Therapy	Lgth of Aqeous a1-PI Therapy	Therapy Response Time			(Stage II) Maint/ enance Therapy	Relapse Rate
1	54/F	Digits and palms had erythematous, edematous, pruritic ulcerated and fisured Lesions. Open wounds were both weeping Bleeding. Antecubital and popliteal fossae were eczematoid and Lichenified. Decreased range of motion of hands.	4 years Oral Prednisone IM Kenalog High Potency Top. Steroids Antibiotics Antipruritics Moisturizer	45 days	Ipain & Pruritis 30 Minutes Irange of motion 24 hours reepith= Day 3 ulcer heal= Day 14			5% cream for 60 days 5% cream & steroid (topical) 47 days No therapy 40 days	0 0 0
2	36/F	Digits and palms were blistering, pruritic, oozing and bleeding. Decreased range of motion in both hands. Left hand had concomitant Lymphangitis with flares of her dermatitis. Mild blistering lesions of feet.	5 years Oral Prednisone IM Kenalog High Potency Top. Steroids Antibiotics Coal Tar Preps Antipuruitics Moisturizers	60 days	Ipain & pruritis 30 Minutes Irange of motion 24 hours Denude/Exfo l= Day 3 Ulcer heal= Day 30			5% cream 21 days 5% cream • steroid 35 days No therapy 50 days	0 0 0
3	36/M	Dorsum of hard had blistering, Weeping, erythematous, edematous and pruritic Lesions. Occasional involvement of chest and arms. Lesions would also go through cycles of crusting.	3 years Oral Prednisone IM Kenalog Antipruritics High Potency Top. Steroids	14 days	Ipain & pruritis 30 Minutes Ieryth= Day 2 Appear Norm= Day 12			No Therapy 90 days	0

4	34/M	Single Chronic Erythematous, Blistering, scalding and pruritic Lesion on right forearms.	5 years Oral Prednisone Antipruritics High Potency, Topical Steroids	42 days	↓pain & pruritis 3 days ↓erythema day 4 Normal appearing skin day 6			5% cream 30 days No Topical Steroids No Therapy 20 days	0 •
5	32/M	Left hand involvement with fissuring, pruritis, scaling, minimal erythema and edema and decreased range of motion.	10 years Oral Prednisone Moisturizers High Potency Topical Steroids	30 days	↓pain & pruritis 30 Minutes ↑range of motion 24 hours Healed skin 30 days			5% cream No Topical Steroids No Therapy 20 days	0 •
6	16/M	Bilateral hand involvement with extensive disease to distal phalanges: fissuring, bleeding, painful and pruritic lesions. Decreased range of motion of hands.	8 years Oral Prednisone Moisturizers Coal Tar Preps	35 days	↓pain & pruritis 4 days ↓erythema 7 days fissures healed day 7			5% cream & Topical Steroids 40 days	0
		* -Only lab data that falls outside of normal limits is tabulated							

WE CLAIM:

1. A method for the prophylaxis or direct treatment of mast cell implicated diseases or injury in mammals which comprises administering to the site of the disease or injury an effective amount of at least one human serine protease inhibitor, its analog, salt or derivative which inhibit the degranulation of mast cells and/or has an affinity to the mediators of mast cells.

2. The method of claim 1 wherein said serine protease inhibitor a natural or recombinant product is selected from the group consisting of alpha 1-antitrypsin, alpha 1-antichymotrypsin, secretory leucocyte protease inhibitor, C-reactive protein, serum amyloid A protein, alpha 2-macroglobulin, eglin, elasnin 3 and elastinal.

3. The method of claim 1 wherein said mast cell implicated disease is a skin disease.

4. The method of claim 5 wherein said disease is allergic or non-allergic rhinitis.

5. The method of claim 1 wherein said disease is arthritis.

6. The method of claim 1 wherein said mediators comprise neutrophils, basophils or eosinophils.

7. The method of claim 1 wherein said mediators comprise cathepsin G and elastase.

8. The method of claim 1 wherein said treatment

includes the administration of a corticosteroid.

9. The method of claim 1 wherein T-cell mediators are inhibited.

10. The method of claim 1 wherein said mast cell implicated diseases is a pulmonary inflammation and alpha-1-antitrypsin is administered by inhalation.

11. A pharmaceutical composition for topical treatment of a patient suffering from a mast cell implicated disease comprising the combination of an effective amount of at least one human type serine protease inhibitor, and a substantially non-aqueous pharmaceutically acceptable carrier.

12. The composition of claim 11 including an effective amount of a corticosteroid.

13. A pharmaceutical composition in inhalation form for treatment of a mast cell implicated pulmonary disease comprising alpha 1-antitrypsin and an inert propellant.

14. A pharmaceutical composition for treating a patient suffering from a mast cell implicated disease comprising an effective amount of at least one human serine protease inhibitor and a suitable carrier.

15. The composition of claim 14 including an effective amount of a corticosteroid.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06847

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/64, 31/56
USCL: 514/8, 12, 21; 552/588,577

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	514/8, 12, 21; 552/577,588

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

APS: Dialog One search- Medline, Biosis, etc/

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Biochemical Medicine and Metabolic Biology,	1-17
Y	Volume 38, issued 1987, Fukusen et al, "Kinetic Studies on the Inhibitions of Mast Cell Chymase by Natureal Serine Protease Inhibitiors: Indications for Potential Biological Functions of these Inhibitors" pp165-169, see entire documents.	1-15
Y	J Clin, Invest., Vol 71, issued June 1983, Schleimer et al, "Effects of Dexamethasone on Mediator Release from Human Lung Fragments and Purified Human Lung Mast Cells", pp 1830-1835, see entire document.	1-15
Y	Am. Rev. Respir. Dis, vol. 135, suppl., issued 1987, Wasserman, "The Regulation of Inflammatory mediator Production by Mast Cell Products," pp S46-S48, see entire document.	1-15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 February 1992	24FEB 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Choon P. Koh

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Clinical Immunology and Immunopathology, Vol. 50, suppl., issued 1989, Marone et al, "Pathophysiology of Human Basophils and Mast Cells in Allergic Disorders," pp.S24-S40, see entire document.	1-15
Y	Annals of Allergy, Vol. 63, No. 6, Suppl. issued 1989, Wasserman, "Mast Cell-mediated inflammation in Asthma," pp. S46-S50, see entire document.	1-15
Y	Ann. Rev. Immunol., Vol. 1, issued 1983, Larsen et al, "Mediators of Inflammation," pp335-359, see entire document.	1-15

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 37/64, 31/56

A1

(11) International Publication Number: WO 92/06706

(43) International Publication Date: 30 April 1992 (30.04.92)

(21) International Application Number: PCT/US91/06847

(22) International Filing Date: 26 September 1991 (26.09.91)

(30) Priority data:

598,241	16 October 1990 (16.10.90)	US
643,727	18 January 1991 (18.01.91)	US
683,620	11 April 1991 (11.04.91)	US

(71)(72) Applicants and Inventors: LEZDEY, John [US/US]; 976 Kingston Drive, Cherry Hill, NJ 08034 (US). WACHTER, Allan [US/US]; 9822 South Grandview, Tempe, AZ 85284 (US).

(74) Common Representative: LEZDEY, John; Suite 400, 400 Market Street, Philadelphia, PA 19106 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), BF (OAPI patent), BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), ML (OAPI patent), MR (OAPI patent), NL (European patent), NO, PL, SE (European patent), SN (OAPI patent), SU+,TD (OAPI patent), TG (OAPI patent).

Published
With international search report.
With amended claims.

Date of publication of the amended claims:
29 May 1992 (29.05.92)

(54) Title: TREATMENT OF INFLAMMATION

(57) Abstract

A method for the prophylaxis or direct treatment of mast cell implicated diseases or injuries in a patient which comprises administering to the site of the disease or injury an effective amount of at least one serine protease inhibitor, its salts, derivatives or analogs which bind with the mediators of mast cells or T-cells.

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU+	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

WO 92/06706 PCT/US91/06847

AMENDED CLAIMS

[received by the International Bureau on 15 April 1992 (15.04.92);
original claims 1-15 replaced by amended claims 1-17 (3 pages)]

1. A method for the prophylaxis or direct treatment of mast cell implicated diseases or injury in mammals which comprises administering to the site of the disease or injury an effective amount of at least one human serine protease inhibitor selected from the group consisting of alpha 1-antitrypsin, secretory leucocyte protease inhibitor, C-reactive protein, serum amyloid A protein and alpha 2-macroglobulin, its analog, salt or derivative which inhibit the degranulation of mast cells and/or has an affinity to the mediators of mast cells.

2. The method of claim 1 wherein said serine protease inhibitor is a recombinant product.

3. The method of claim 1 including the addition of an effective amount of alpha 1-antichymotrypsin.

4. The method of claim 1 wherein said mast cell implicated disease is a skin disease and the serine protease inhibitor is topically applied.

5. The method of claim 1 wherein said disease is allergic or non-allergic rhinitis and the serine protease inhibitor is applied nasally.

6. The method of claim 1 wherein said disease is arthritis.

7. The method of claim 1 which comprises administering an effective amount of a serine protease inhibitor which inhibits T-cell lymphokine glycosylation enhancing factor.

8. The method of claim 1 which includes the administration of synergistically effective amounts of a corticosteroid.

9. The method of claim 1 wherein said mast cell implicated diseases is a pulmonary inflammation and microcrystalline alpha-1-antitrypsin is administered by inhalation.

10. A method for the treatment of mast cell implicated diseases in mammals which comprises administering to the site of the disease an effective amount of alpha 1-antichymotrypsin.

11. A pharmaceutical composition for topical treatment of a patient suffering from a mast cell implicated disease comprising the combination of an effective amount of at least one human type serine protease inhibitor selected from the group consisting of alpha 1-antitrypsin, secretory leucocyte inhibitor, C-reactive protein, serum amyloid A protein and alpha 2-macroglobulin, its analog, salt or derivative and a pharmaceutically acceptable carrier.

12. The composition of claim 11 including an effective amount of a corticosteroid.

13. The composition of claim 10 including an effective amount of alpha 1-antichymotrypsin.

14. The composition of claim 11 wherein said carrier comprises a topical cream.

15. A pharmaceutical composition in inhalation form for treatment of a mast cell implicated pulmonary disease comprising microcrystalline alpha 1-antitrypsin and an inert propellant.

16. A pharmaceutical composition for treating a patient suffering from a mast cell implicated disease comprising an effective amount of alpha 1-antichymotrypsin and alpha 1-

antitrypsin and a suitable carrier.

17. The composition of claim 15 including an effective amount of a corticosteroid.